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From the metabolic syndrome to NAFLD or vice versa?

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Abstract

The metabolic syndrome encompasses metabolic and cardiovascular risk factors which predict diabetes and cardiovascular disease (CVD) better than any of its individual components. Nonalcoholic fatty liver disease (NAFLD) comprises a disease spectrum which includes variable degrees of simple steatosis (nonalcoholic fatty liver, NAFL), nonalcoholic steatohepatitis (NASH) and cirrhosis. NAFLD is the hepatic manifestation of the metabolic syndrome, with insulin resistance as the main pathogenetic mechanism. Recent data indicate that hyperinsulinemia is probably the consequence rather than cause of NAFLD and NAFLD can be considered an independent predictor of cardiovascular disease. Serum free fatty acids derived from lipolysis of visceral adipose tissue are the main source of hepatic triglycerides in NAFLD, although hepatic de novo lipogenesis and dietary fat supply contribute to the pathogenesis of NAFLD. Approximately 10–25% NAFLD patients develop NASH, the evolutive form of hepatic steatosis. Presumably in a genetically predisposed environment, this increased lipid overload overwhelms the oxidative capacity and reactive oxygen species are generated, leading to lipid peroxidation, cytokine induction, chemoattraction of inflammatory cells, hepatic stellate cell activation and finally fibrogenesis with extracellular matrix deposition. No currently available therapies for NAFLD and NASH exist. Recently nuclear receptors have emerged as key regulators of lipid and carbohydrate metabolism for which specific pharmacological ligands are available, making them attractive therapeutic targets for NAFLD and NASH.

Abbreviations

ALT, alanine aminotransferase; BMI, body mass index; CVD, cardiovascular disease; FFA, free fatty acids; HDL, high density lipoprotein; 1H-MRS, proton magnetic resonance spectroscopy; IDF, International Diabetes Federation; LDL, low density lipoprotein; NAFLD, nonalcoholic fatty liver disease; SNP, single nucleotide polymorphism; VLDL, very low density lipoprotein

1. Introduction

The metabolic syndrome is a cluster of metabolic and cardiovascular risk factors which predicts diabetes and cardiovascular disease (CVD) better than any of its individual components [1]. The latest definition of the metabolic syndrome by International Diabetes Federation (IDF) includes abdominal obesity defined by increased waist circumference (≥ 94 cm in men and ≥ 80 cm in women) and two or more of the following features: elevated blood pressure, fasting glucose or triglyceride concentrations, or low HDL cholesterol [1]. The term nonalcoholic fatty liver disease (NAFLD) comprises a disease spectrum which includes variable degrees of simple steatosis (nonalcoholic fatty liver, NAFL), nonalcoholic steatohepatitis (NASH) and cirrhosis [2] and [3]. NAFLD can be considered the hepatic manifestation of the metabolic syndrome. Simple steatosis is benign, whereas NASH is defined by the presence of hepatocyte injury, inflammation and/or fibrosis which can lead to cirrhosis, liver failure and hepatocellular carcinoma. In contrast to the metabolic syndrome, the definition of nonalcoholic fatty liver disease (NAFLD) includes only one component: liver fat content >5 – 10% by weight in the absence of excess alcohol consumption or any other liver disease. This review discusses the pathogenetic link between insulin resistance, the metabolic syndrome and fat accumulation in the liver (NAFLD), and the mechanisms leading to the shift from NAFLD to NASH.

2. Pathogenesis of NAFLD

2.1. NAFLD and hepatic insulin resistance

The liver is the main site of insulin action, in addition to skeletal muscle and adipose tissue [4]. In the fasting state, insulin restrains hepatic glucose production which maintains plasma glucose concentration normal. When the liver gets fatty because of NAFLD, the ability of insulin to inhibit hepatic glucose production is impaired, when measured directly using a low-dose hyperinsulinemic euglycemic clamp combined with a glucose tracer [5] and [6]. This hepatic insulin resistance leads to a slight increase in plasma glucose concentrations and stimulation of insulin secretion. Hyperinsulinemia is likely to be the consequence rather than a cause of NAFLD, since exogenous insulin therapy for 7 months decreases significantly liver fat content [7]. Hyperinsulinemia could contribute to hypertension by stimulating basal sympathetic tone [7] and [8] and renal sodium reabsorption [10].

Another action of insulin is to restrain production of VLDL [11]. Hepatic insulin resistance is characterized by a defect in insulin inhibition of VLDL production. In subjects with NAFLD, the liver overproduces triglyceride-rich VLDL in the fasting state [12] and [13] and during hyperinsulinemia [14]. This leads to hypertriglyceridemia and a low HDL cholesterol concentration [15] and [16].

2.2. NAFLD vs. the metabolic syndrome in CVD

The metabolic syndrome increases the risk of CVD approximately twofold [17]. Recently, several prospective epidemiological studies have shown that both increased serum liver enzyme concentrations [18] and [19] and hepatic steatosis determined by ultrasound [20] and [21] are able to predict development of CVD independent of alcohol consumption or traditional CVD risk factors, such as serum LDL cholesterol concentrations. In a Japanese prospective study, NAFLD predicted CVD better than the metabolic syndrome [20]. Multivariate analyses suggested that NAFLD but not the metabolic syndrome was an independent predictor of CVD [20].

2.3. NAFLD vs. the metabolic syndrome in type 2 diabetes

Subjects with the metabolic syndrome have a four- to fivefold increased risk of type 2 diabetes as compared to those without the syndrome. Several studies have also shown that increased serum liver enzyme concentrations, the most common cause of which is NAFLD in the general population [22], predict type 2 diabetes independent of obesity [23]. An elegant study by Sattar et al. showed that prior to the onset of type 2 diabetes, fasting plasma glucose, serum ALT, and triglyceride concentrations increase in the absence of changes in body weight [24]. Type 2 diabetic patients have on the average 80% more liver fat than carefully age- and gender-matched equally obese non-diabetic subjects [25]. These data suggest that hepatic fat accumulation is involved in the pathogenesis of type 2 diabetes.

2.4. Pathogenesis of NAFLD: recent insights of human studies (Fig. 1)

2.4.1. Adipose tissue

Adipose-tissue insulin resistance, defined as insulin resistance of lipolysis, is associated with increased liver fat content independent of obesity in humans [13], [26] and [27]. According to stable isotope studies, serum FFA are the main source of hepatic triglycerides in NAFLD [28]. Adipose-tissue insulin resistance of lipolysis is therefore likely to contribute to fat accumulation in the liver. In humans, PPAR γ agonist (thiazolidinedione) treatment significantly decreases liver fat content by approximately 40%. This decrease is closely correlated with an increase in the serum concentrations of the adipose-tissue-derived hormone adiponectin [29]. In mice, the main target of adiponectin action is the liver, where it decreases insulin resistance [30]. In human fatty liver, gene expression of PPAR γ is unaltered and that of PPAR γ 2 up-regulated [31]. Therefore, the decrease in the liver fat by PPAR γ agonists cannot be mediated via liver PPAR γ but might be mediated by circulating adiponectin. Adipose tissue has been shown to be inflamed in subjects with high as compared to

those with low liver fat content independent of obesity [32] and [33]. This inflammation is characterized by increased macrophage accumulation and increased gene expression of macrophage markers, such as CD68, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 α [32] and [33]. Analysis of lipid composition revealed that the inflamed adipose tissue in subjects with high as compared to those with low liver fat content contains more ceramides [33]. Ceramides are sphingolipids with a variety of metabolic functions, such as mediation of saturated fat-induced insulin resistance [34] and [36], inflammation, and regulation of apoptosis [37] and [38]. Although the mechanisms by which hepatic fat accumulation associates with adipose-tissue inflammation remain to be determined, macrophage infiltration and increased ceramide concentrations could contribute to adipose-tissue insulin resistance in NAFLD [39] and [40].

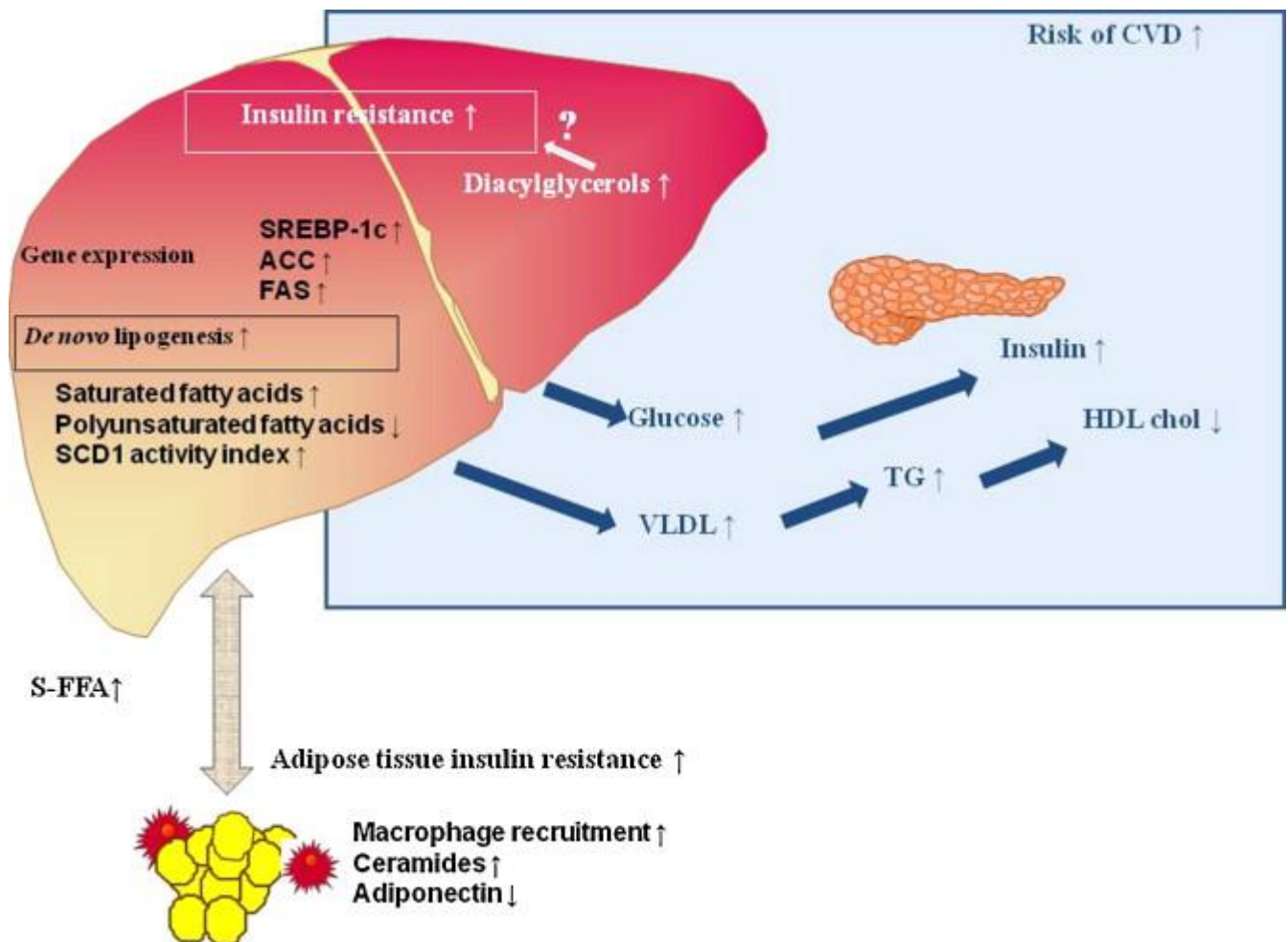


Fig. 1.

A summary of pathogenic mechanisms, associated conditions and consequences of hepatic fat accumulation due to nonalcoholic causes. Please see text for references.

2.4.2. De novo lipogenesis

In humans, de novo lipogenesis produces saturated fatty acids [41] and [42]. Hepatic de novo lipogenesis is significantly increased in subjects with NAFLD, when measured using stable isotopes [28]. Hepatic gene expression of sterol regulatory element-binding protein (SREBP) 1c, key transcriptional activator of lipogenic genes, as well as of acetyl-CoA carboxylases (ACCs) and fatty acid synthase (FAS) have been shown to be increased in subjects with as compared to those without NAFLD [43]. Analyses of fatty acid composition revealed that there is an excess of saturated fatty acids and saturated fatty acids –containing triglycerides in the human fatty liver [44]. In addition, the activity of a lipogenic enzyme stearoyl-CoA desaturase 1 (SCD1) is increased and the

percentage of long polyunsaturated fatty acids decreased in subjects with as compared to those without NAFLD [44]. This could decrease the ability of long polyunsaturated fatty acids to inhibit SREBP-1c activity [45] and [46].

2.4.3. Hepatic lipids and insulin resistance

The mechanisms underlying hepatic insulin resistance in human NAFLD have remained poorly characterized. We have recently shown that hepatic concentrations of diacylglycerols, the immediate precursors of triglycerides [47], but not of ceramides are directly related to hepatic fat content in the human liver [44]. Since both ceramides and diacylglycerols are able to cause insulin resistance by interfering with insulin signalling pathway [39] and [48], diacylglycerols rather than ceramides might contribute to hepatic insulin resistance in human NAFLD.

2.5. Causes of hepatic fat accumulation

2.5.1. Acquired factors

Modest weight loss of 5–10% of body weight decreases liver fat by 40–80% in non-diabetic subjects and type 2 diabetic patients [49] and [51]. Weight gain of ~10% by overfeeding of fast-food and sedentary lifestyle in 18 young healthy subjects has been shown to increase liver fat 2.5-fold in 4 weeks [52]. In cross-sectional studies, subjects with NAFLD have been shown to consume more fat, especially saturated fat [53] and [54] than subjects without NAFLD. Also, the intake of polyunsaturated fat has been shown to be lower in subjects with NAFLD than in those without in some [54] and [55] but not all [56] studies. Subjects with NAFLD tend to consume products with high glycemic index [57] and more soft drinks [56] as compared to subjects with a normal liver fat content.

2.5.2. Genetic factors

Data are emerging to suggest that genetic factors contribute to variation in liver fat content. A Danish twin study suggested heritability of serum ALT concentrations to be 33–48% independent of BMI and alcohol consumption [58]. We have recently confirmed this finding in a Finnish cohort of 313 twins, in which the heritability of serum ALT concentrations was 55% [59]. In the Dallas Heart Study, in which liver fat content was measured quantitatively using ¹H-MRS, a genome-wide association scan revealed that the rs738409 SNP in adiponutrin gene is strongly associated with increased liver fat content ($p = 3.4 \times 10^{-4}$ in European Americans, $p = 7.5 \times 10^{-9}$ in African Americans, and $p = 2.0 \times 10^{-10}$ in Hispanics [60]. The other 9228 genetic variants analysed were not significantly associated with liver fat content [60].

3. Pathogenesis of NASH

Only approximately 10–25% of patients with NAFLD develop NASH [63] and [64]: the factors responsible for the switch from steatosis to steatohepatitis have been subjects of extensive investigation and speculation, but currently remain elusive. The proposed “two-hits” model by Day and James [65] was the first attempt to provide a pathophysiological rationale to the progression of liver damage, claiming that the reversible intracellular deposition of triacylglycerols (TAG; “first hit”) leads to metabolic and molecular alterations that sensitize the liver to the “second hit”, usually referred to as oxidative stress and cytokine-induced liver injury. Although quite simplistic in view of more recent data, this model can nevertheless provide a framework to revise the main mechanisms involved in the pathogenesis of hepatic steatosis and its progression to steatohepatitis.

3.1. “First hit”: from normal to fatty liver (Fig. 2)

As stated before, insulin resistance is thought to be inevitably linked to the pathogenesis of NAFLD [66]. This condition classically involves multiple sites: the muscle, where it decreases glucose uptake and utilisation, the liver, where it is responsible for the overproduction of glucose despite fasting hyperinsulinaemia, and the adipose tissue, where lipolysis is not adequately suppressed by

insulin, with subsequent release of glycerol and non-esterified fatty acids (NEFA) into the bloodstream. In addition to increased FFA efflux to the liver and increased hepatic *de novo* lipogenesis, another important source of hepatic TAG is represented by exogenous lipids. During the post-prandial phase, dietary lipids are transported from the gut into the bloodstream in the form of chylomicrons and stored in the liver, where they are processed and assembled with apolipoprotein B 100 (apoB100) to form very low density lipoproteins (VLDL). In overweight non-diabetic subjects, a high-fat diet is able to promote a 35% increase of liver fat content in only 10 days [67]. In subjects with NAFLD, dietary fat supply accounts for about 15% of intrahepatic lipid accumulation [28].

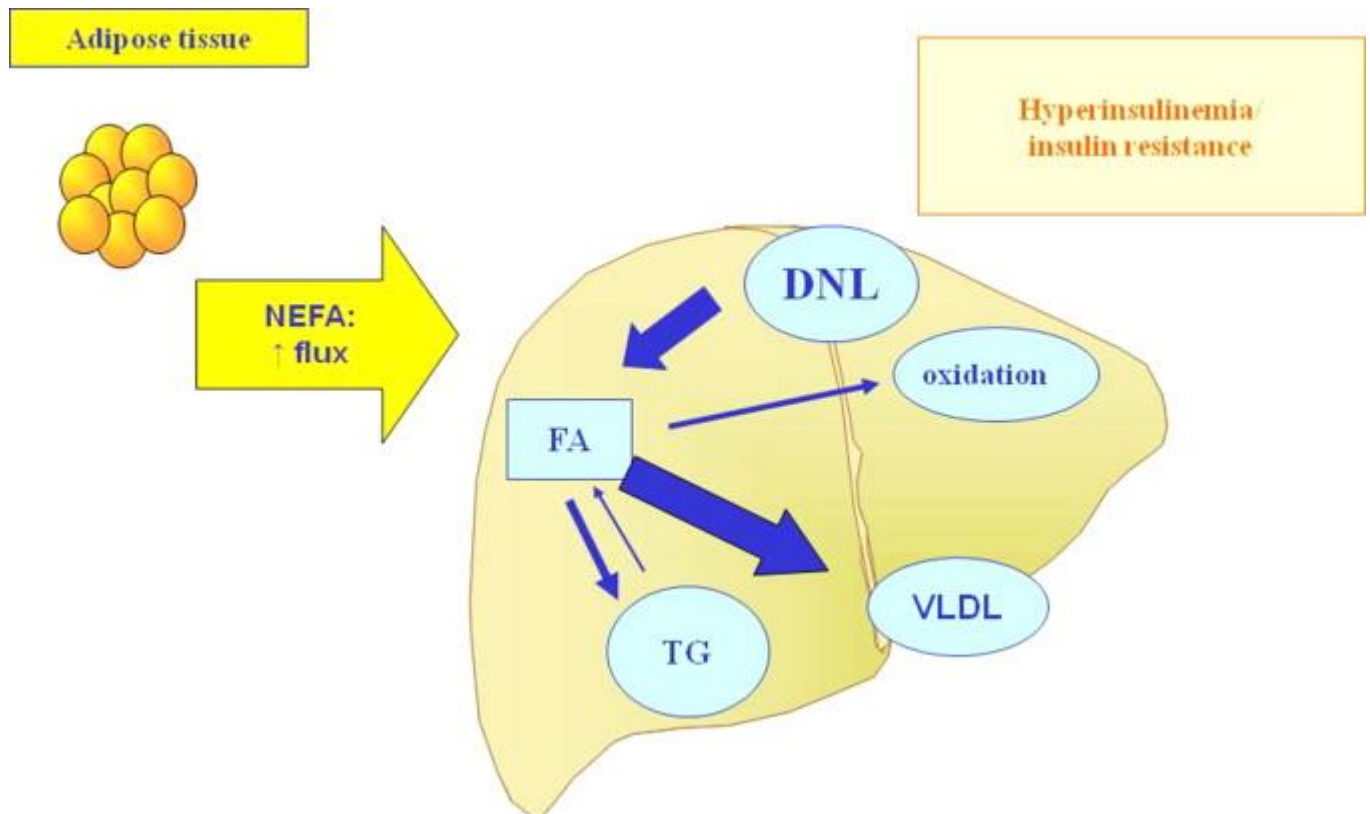


Fig. 2.

Role of fatty acids in the pathogenesis of fatty liver. In NAFLD the ability of insulin to suppress lipolysis is impaired, leading to an increased supply of FFA to the liver; *de novo* synthesis of fatty acids is increased, being responsible for almost 25% of the increased liver fat content. The fatty acid oxidation is up-regulated, but the increase in intrahepatic fatty acid availability exceeds the liver's ability to oxidize excess fatty acids. Finally, subjects with NAFLD have increased rates of hepatic VLDL-triglycerides (TG) secretion, but a failure to increase adequately the secretion rate of apoB100, which limits the liver capacity to export TG. NEFA: non-esterified fatty acids; TG: triglycerides; VLDL: very low density lipoproteins; DNL: *de novo* lipogenesis.

Although the liver is the major organ for lipid distribution, the hepatic ability to store fat is limited and the lipid excess is either oxidized or released as VLDL. An increase in intrahepatic fat content leads to an up-regulation of oxidative mechanisms also in NAFLD patients [68], but NEFA are oxidized less efficiently because of mitochondrial uncoupling [69]. Subjects with NAFLD have increased rates of hepatic VLDL-triglycerides (TG) secretion, the major source of circulating TG, which is responsible for the increase in serum TG concentrations commonly observed in patients. However, the liver capacity to export TG is limited by an inadequate increase of the secretion rate of apoB100, which leads to a dissociation in VLDL-TG and VLDL apoB100 kinetics [13].

3.2. “Second hit”: from steatosis to steatohepatitis (Fig. 3)

The progression from steatosis to steatohepatitis is likely due to the intersection of multiple mechanisms, probably superimposing on a genetic susceptibility to metabolic and liver damage.

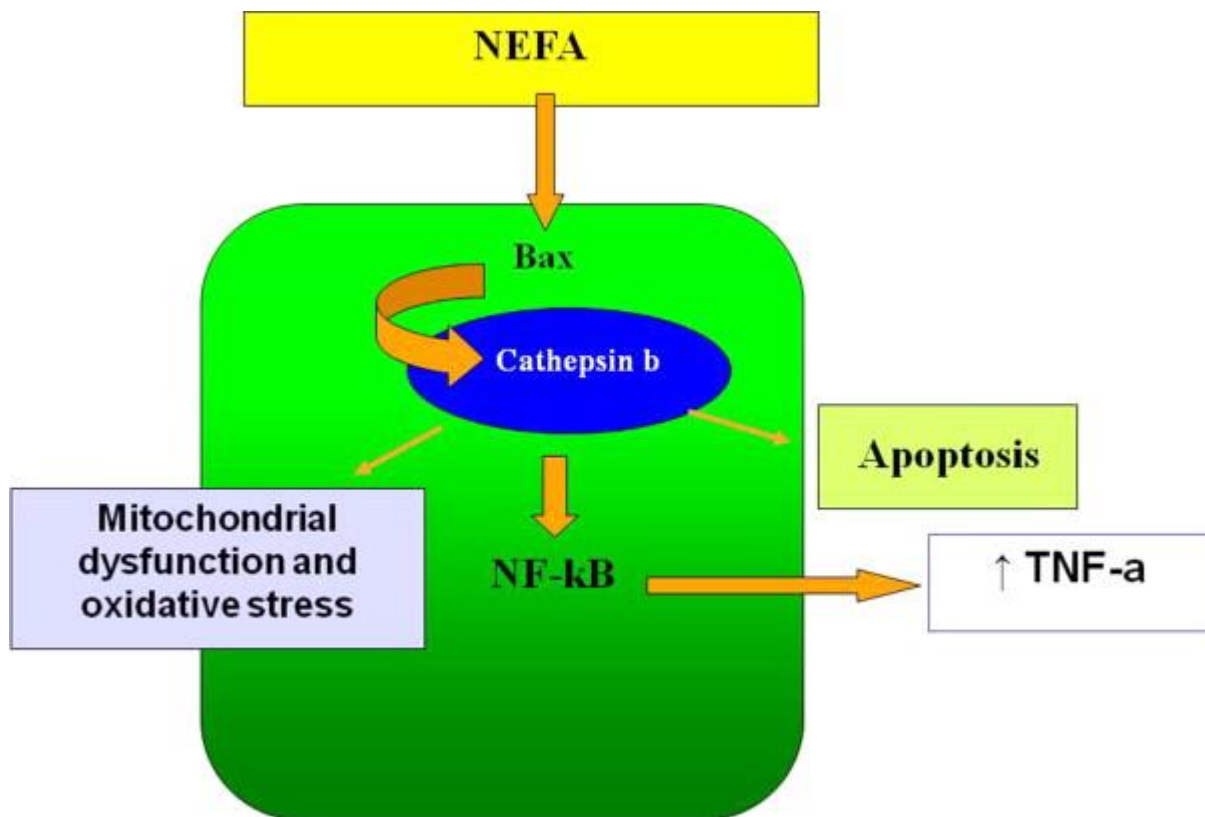


Fig. 3.

NEFA induce mitochondrial dysfunction, oxidative stress, TNF- α expression and apoptosis through lysosomal destabilisation. NEFA: non-esterified fatty acids; TNF- α : tumour necrosis factor-alpha.

Oxidative stress is increased in patients with NASH [70]. An excess of NEFA supply to the liver increases mitochondrial and peroxisomal β -oxidation, promotes microsomal induction of CYP4A1 and CYP2E1 and leads to elevated production of reactive oxygen species (ROS) [71]. Increased levels of ROS contribute to organelle toxicity by an increase in lipid peroxidation with subsequent inflammation and fibrosis.

Mitochondrial dysfunction and other organelle stress are observed in hepatocytes of subjects with steatosis. In particular, structural abnormalities in the mitochondria (megamitochondria, paracrystalline inclusion bodies) [69] are more frequently observed in NASH than in NAFL. Moreover, the activity of the mitochondrial respiratory chain complexes is markedly decreased in patients with NASH leading to an inefficient ATP generation [72].

Although hepatic steatosis has long been considered the main cause of inflammation and liver damage, intracellular triglyceride storage in the liver might actually represent a mechanism to entrap NEFA in an inactive form, thus limiting the NEFA-induced lipid peroxidation and oxidative stress. This has been recently confirmed in an animal model, where the knockdown of diacylglycerol acyltransferase decreased hepatic steatosis by inhibiting the final step in triglyceride synthesis, but increased hepatic fat content, up-regulated CYP2E1, exacerbated hepatic oxidative damage and increased liver inflammation and fibrosis [73]. This study emphasizes the concept of lipotoxicity, which refers to cellular dysfunction induced by an overload of NEFA in ectopic tissues, such as muscle and liver. Lipotoxicity appears to be a key factor in the progression of hepatic steatosis to steatohepatitis by inducing an increased expression of proinflammatory cytokines, an inhibition of mitochondrial β -oxidation, elevated production of ROS as well as an

enhanced generation of toxic lipid intermediates [74]. NEFA are also able to induce apoptosis in the hepatocyte [75]; compared to patients with simple steatosis, subjects with NASH show higher rate of apoptosis in the liver. Induction of the proapoptotic pathway is mediated by up-regulation of Fas [76], activation of Jun N-terminal kinase [75] and successive destabilisation of lysosomes [77]. Lysosomes have been recently proposed as primary targets of the lipotoxic effects. In an animal model, intrahepatic lipid accumulation by a high-fat diet induced translocation of the proapoptotic factor Bax to the lysosome and release of lysosomal cysteine protease cathepsin B, leading to activation of NF- κ B, increased transcription of TNF- α , increased ROS production and mitochondrial dysfunction [77] and [78] (Fig. 3).

Other key players in the pathogenesis of steatosis and the progression to steatohepatitis are the so called adipokines (adiponectin, leptin, and resistin) and several cytokines (such as TNF- α , IL-6, and IL-1 β) secreted by the adipocyte or by the inflammatory cells that infiltrate the adipose tissue in insulin-resistant states. Visceral adipose tissue represents a preferential source of adipokines and cytokines potentially acting on the liver tissue. The adipokines exert both metabolic and immune functions and mediate the metabolic cross-talk between adipose tissue, muscle and liver. Some of them, as adiponectin, possess anti-inflammatory and anti-inflammatory properties, while others, as TNF- α and leptin, have an opposite action. The liver damage in NASH could result from an imbalance between pro- and anti-inflammatory adipokines. In fact, adiponectin plasma levels are decreased in NAFLD patients and inversely related to hepatic insulin resistance, hepatic fat content [79], degree of inflammation [80] and extent of fibrosis in the liver [81]. High TNF- α and low adiponectin plasma levels have been indicated as independent predictors of NASH in NAFLD patients [80].

On the contrary, serum levels of resistin are increased in patients with NASH and correlated with hepatic insulin resistance, hepatic fat content [82] and the histological inflammatory grade [83].

Leptin is a key mediator in the development of insulin resistance, hepatic steatosis and liver damage. In patients with NASH serum leptin levels were positively correlated with hepatic fat content, fibrosis and inflammation, as well as with serum lipids, glucose, insulin, C-peptide and ALT [84]. Leptin down-regulates insulin signalling in the liver [85], and favours the development and progression of fibrosis [86].

Cytokines, particularly TNF- α , IL-6, and IL-1 β , are involved in the recruitment and activation of Kupffer cells and the transformation of stellate cells into myofibroblasts, both of which contribute to the progression from steatosis to steatohepatitis. They can also affect insulin signalling, thus playing a role in the development of insulin resistance [87].

Insulin resistance per se and the subclinical inflammatory state usually associated with it might have a direct role in the pathogenesis of liver injury. Hyperglycemia and hyperinsulinemia can cause an up-regulation of the tissue connective growth factor, thus promoting fibrogenesis [88]. In obese, diabetic rats a high-fat diet enhances insulin resistance and leads to severe steatohepatitis via an up-regulation of genes for lipogenesis (sterol regulatory element-binding protein-1c, SREBP-1c, fatty acid synthase, FAS), inflammation (IL-6, TNF- α) and fibrogenesis (transforming growth factor-beta, TGF- β) [89].

3.3. Predictive factors of advanced fibrosis in NAFLD

Various clinical and laboratory markers were shown to be associated with advanced fibrosis in patients with NAFLD (Table 1). The need of a non-invasive test able to accurately distinguish NASH from NAFL has led several investigators to combine some clinical and laboratory variables in order to create surrogate markers of advanced disease. By analysing a large cohort of biopsy-

proven NAFLD subjects, Angulo et al. generated a NAFLD fibrosis score composed of six readily available variables (age, BMI, hyperglycemia, platelet count, albumin, AST/ALT ratio), to discriminate patients with or without severe fibrosis. This score accurately predicts the presence or the absence of advanced fibrosis in about 75% of NAFLD patients [90]. More recently, the European Liver Fibrosis study group validated the ELF score, made up of a combination of three components of the extracellular matrix (serum hyaluronic acid, amino-terminal propeptide of type III collagen and tissue inhibitor of metalloproteinase 1). This algorithm has shown a good performance in differentiating fibrosis stages in NAFLD, with a better accuracy when combined with the NAFLD fibrosis score (over 90% in distinguishing severe fibrosis) [91]. However, all these scores are not still employed in routine clinical practice.

Table 1.

Predictive factors for advanced fibrosis in retrospective and follow-up studies, respectively.

	Retrospective (n = 17)	Follow-up (n = 5)
Obesity/BMI/weight gain	7	3
Diabetes/high glucose	5	2
High insulin/insulin resistance/metabolic syndrome	10	2
Ferritin	2	–
Age	3	1
High triglycerides	2	–
Male gender	1	–
Female gender	1	–
ALT > 2× or AST/ALT > 1	7	1

3.4. Nuclear receptors in the pathogenesis of NAFLD and NASH

Homeostatic regulation of lipid metabolism requires cellular sensors that can monitor the concentration of bioactive lipids and coordinate the enzymatic cascades that regulate lipid synthesis and catalysis. On this regard, the nuclear receptors are transcription factors that serve as intracellular sensors for endocrine hormones and dietary lipids, and have emerged as key regulators of lipid and carbohydrate metabolism [93] and [94]. In contrast to extracellular receptors, which bind to peptide ligands (e.g., growth factors and insulin) at the cell surface and activate cytoplasmic kinase cascades, nuclear receptors interact directly with lipophilic ligands and regulate expression of target genes. Nuclear receptors function as ligand-dependent transcription factors by binding to specific DNA sequences called response elements within the regulatory regions of target-gene promoters and activate target-gene expression [93].

The retinoid X receptor (RXR) represents a common binding partner for other nuclear receptors to form an heterodimer. The ability of RXR agonists to regulate target genes of multiple permissive partners implies that in vivo such compounds may have pharmacologic use as panagonists of several metabolically important pathways, although this advantage is likely to be offset by poor selectivity. In addition, the propensity of RXR agonists to induce hypertriglyceridemia in animals and humans indicates that targeting the heterodimeric partners of RXRs is likely to result in more suitable candidates for drug therapy [93].

3.5. Farnesoid X receptor (FXR), lipid metabolism and chronic liver disease

FXR is a bile acid sensor that acts as an RXR heterodimer and functions to protect cells and organs, especially the liver and the intestine, against bile acid toxicity [95], [96] and [97]. Bile acids are the major ligands for FXR, and by activating FXR have a variety of transcriptional effects, many of which are geared toward preventing synthesis and uptake and promoting excretion of bile acids. One effect of FXR activation is downregulation of Cyp7A1 and thus bile acid synthesis, accomplished through induction of SHP (short heterodimer partner) which then represses Cyp7A1 transcription. A second effect of FXR activation is downregulation of the hepatic bile acid import

pumps NTCP and OATP (Na-dependent taurocholate cotransporting polypeptide and organic anion transporting polypeptide), thus reducing the import of bile acids from the plasma compartment into the hepatocyte. A third effect of FXR activation is up-regulation of the hepatic bile acid export pumps BSEP and MRP2 (bile salt export protein and multidrug resistance-associated protein-2), thus increasing the export of bile acids out of the hepatocyte into the bile. A fourth effect of FXR activation is the induction of pathways responsible for detoxification of bile acids.

It has been known for many years that the bile acid pool size has a profound effect on lipid metabolism. The reduced bile acid pool, following either administration of bile acid binding resins (e.g., cholestyramine or cholestipol) or ileal surgery, results in reduced levels of plasma LDL and increased plasma triglyceride and HDL. The finding that administration of bile acids (CDCA or CA) to humans or animals results in reduced plasma triglyceride and HDL levels and increased LDL is entirely consistent with a key role for bile acids in controlling plasma lipids [98].

Studies with *Fxr*^{-/-} mice or following administration of FXR-specific agonists have demonstrated that FXR plays a central role in controlling lipid homeostasis, by reducing both hepatic lipogenesis and plasma triglyceride and cholesterol levels. FXR activation induces genes involved in lipoprotein metabolism/clearance and represses hepatic genes involved in the synthesis of triglycerides. For example, treatment of mice with FXR agonists results in the repression of *Srebp-1c* mRNA levels in murine livers or isolated murine primary hepatocytes [99] and [100]. This repression was not observed with *Shp*^{-/-} mice, suggesting that *SHP*, a known FXR target gene, is required for the repression [99]. Since *SREBP-1c* functions as a critical transcription factor that regulates many genes involved in both fatty acid and triglyceride synthesis [94], it is not surprising that hepatic triglyceride synthesis and secretion is reduced following the repression of *Srebp-1c* by FXR.

Activation of FXR also results in increased hepatic expression of receptors (VLDL receptor and syndecan-1) that are involved in lipoprotein clearance and in decreased expression of proteins (apoC-III and ANGPTL3) that normally function as inhibitors of lipoprotein lipase [101]. Finally, FXR induces human PPAR α [102], a nuclear receptor that functions to promote fatty acid β -oxidation (see below). Taken together, these data suggest that FXR activation lowers plasma triglyceride levels via both repressing hepatic lipogenesis and triglyceride secretion, and increasing the clearance of triglyceride-rich lipoproteins from the blood.

Nevertheless, the ultimate proof for the FXR pivotal role in lipid homeostasis was offered by the generation of *FXR*^{-/-} mice. These mice accumulate fat in the liver and, together with changes in bile acid homeostasis, they show a pro-atherogenic lipid profile (elevated TG and cholesterol plasma levels) as a direct consequence of the FXR absence [103]. The observation that *Fxr*^{-/-} mice have increased plasma LDL and HDL levels is consistent with an important role for FXR in controlling plasma lipoprotein levels. As stated above, FXR controls cholesterol metabolism mostly through expressional modulation of CYP7A1, the rate-limiting enzyme for cholesterol catabolism into bile acids. When the bile acid pool size increases, FXR activation leads to repressed expression of *Cyp7a1*. Downregulation of CYP7A1 by FXR would lead to decrease cholesterol catabolism with net accumulation of the sterol in the liver and in the plasma. In line with this issue, pharmacological inhibition of FXR by antagonist molecules is a promising approach to enhance cholesterol conversion into bile acids, hence decrease cholesterol levels in plasma. However, the story is not as simple as it seems. FXR knockout mice, in fact, display increased intestinal absorption of cholesterol, suggesting a negative regulatory role for FXR on cholesterol absorption in the intestine [104]. Also, FXR might act as an enhancer of reverse cholesterol transport, the process of cholesterol delivery from peripheral tissues to the liver for biliary disposal [104]. Overall, the role of FXR agonism–antagonism in cholesterol disposal and HDL cholesterol plasma

levels is still controversial, and contradictory data in the literature provide the impetus to address this critical issue in the next future.

Fxr^{-/-} mice show impaired glucose tolerance and insulin sensitivity compared to wild-type mice. Hyperinsulinemic–euglycemic clamp studies reveal that Fxr^{-/-} mice also display insulin resistance in the liver, muscle and white adipose tissue [105] and [106]. Treatment of mice with the FXR agonist GW4064 or cholic acid, or following infection with adenovirus that expresses a constitutively active FXR-VP16 fusion protein, resulted in a significant reduction of plasma glucose levels and improved insulin sensitivity [105], [106] and [107]. These effects were noted in three different diabetic models (db/db, ob/ob or KK-Ay mice). Activation of hepatic FXR regulates gluconeogenesis, glycogen synthesis and insulin sensitivity. Overall, these changes result in decreased hepatic gluconeogenesis, decreased plasma glucose levels and increased hepatic glycogen synthesis. FXR activation also results in increased phosphorylation of hepatic IRS-1 and IRS-2 and increased insulin sensitivity/signalling [100]. The finding that activation of FXR significantly lowered plasma glucose, triglyceride, cholesterol and free fatty acid levels in diabetic mouse models [106], suggests that FXR agonists might prove useful in the treatment of hyperglycemia and hyperlipidemia that are observed in patients with type 2 diabetes.

The concerted actions of FXR on lipid and glucose metabolism indicate that FXR contributes to the coordinated regulation of the shift from the fasted to fed transition by interfering with lipid- and carbohydrate-induced changes in gene expression. In the post-prandial state, BAs secreted into bile and discharged into the intestine stimulate absorption of lipids that form chylomicrons in lymph. Carbohydrates, like glucose, are absorbed and reach the liver via the portal vein. BAs undergo enterohepatic recirculation and return to the hepatocyte where they activate, among other actions, FXR. Activated Fxr subsequently interferes with glycolysis, to stimulate glycogen storage, and inhibits de novo lipogenesis. Finally, FXR inhibits BA synthesis by inhibition of Cyp7a1 expression. Thus, FXR participates in the metabolic adaptation of the hepatocyte in the post-prandial state [108].

As mentioned above, FXR is thought to play a substantial role in liver diseases in light of its ability to affect bile acid homeostasis. A potential role of FXR in chronic cholestatic diseases is suggested by reduced FXR expression observed in patients affected by progressive familial intrahepatic cholestasis type 1. FXR activation could be beneficial in chronic cholestatic disorders by re-establishing a proper bile acid excretion and bile formation and by activating phase 1 and phase 2 detoxifying enzymes. Many of these findings, however, are from experimental animal models that not fully recreate human diseases, thus expectations are yet to be verified [98]. Activation of PXR (that shares similar functions with FXR) resulted not effective in ameliorating cholestatic injury in patients with primary biliary cirrhosis [109]. Activation of FXR by the synthetic agonist GW4064 reduced neutral lipid accumulation in the livers of db/db mice [106]. However, UDCA was not associated with an improvement in serum liver biochemistries or histology when compared with placebo [110].

Exposure to routinely measured concentrations of BAs increases HCV replication by a novel mechanism involving activation of the nuclear-receptor FXR. In addition, HSC exposure to BA concentrations found in chronic cholestatic conditions induces cell proliferation through EGF receptor activation [111]. FXR has been also found to enhance liver regeneration and cell proliferation. If this aspect may be beneficial in certain diseases, a potential tumourigenic effect of FXR needs to be ruled out before considering a long term administration of its agonists to patients with chronic liver diseases [98].

3.6. Peroxisome-proliferator-activated receptors (PPARs)

The peroxisome-proliferator-activated receptors (PPARs) are a subfamily of the nuclear-receptor superfamily and regulate gene expression in response to ligand binding. After ligand binding, PPARs undergo specific conformational changes that allow for the recruitment of one coactivator protein or more.

PPARs regulate gene transcription by two mechanisms. As already mentioned, transactivation is DNA-dependent and involves binding to PPAR response elements of target genes and heterodimerisation with RXR. A second mechanism, transrepression, may explain the anti-inflammatory actions of PPARs. It involves interfering with other transcription-factor pathways in a DNA-independent way [112]. Various fatty acids serve as endogenous ligands for PPARs, and three PPARs, designated PPAR α , PPAR γ , and PPAR δ have been identified to date with different effects in terms of gene expression, as summarized in Fig. 4.

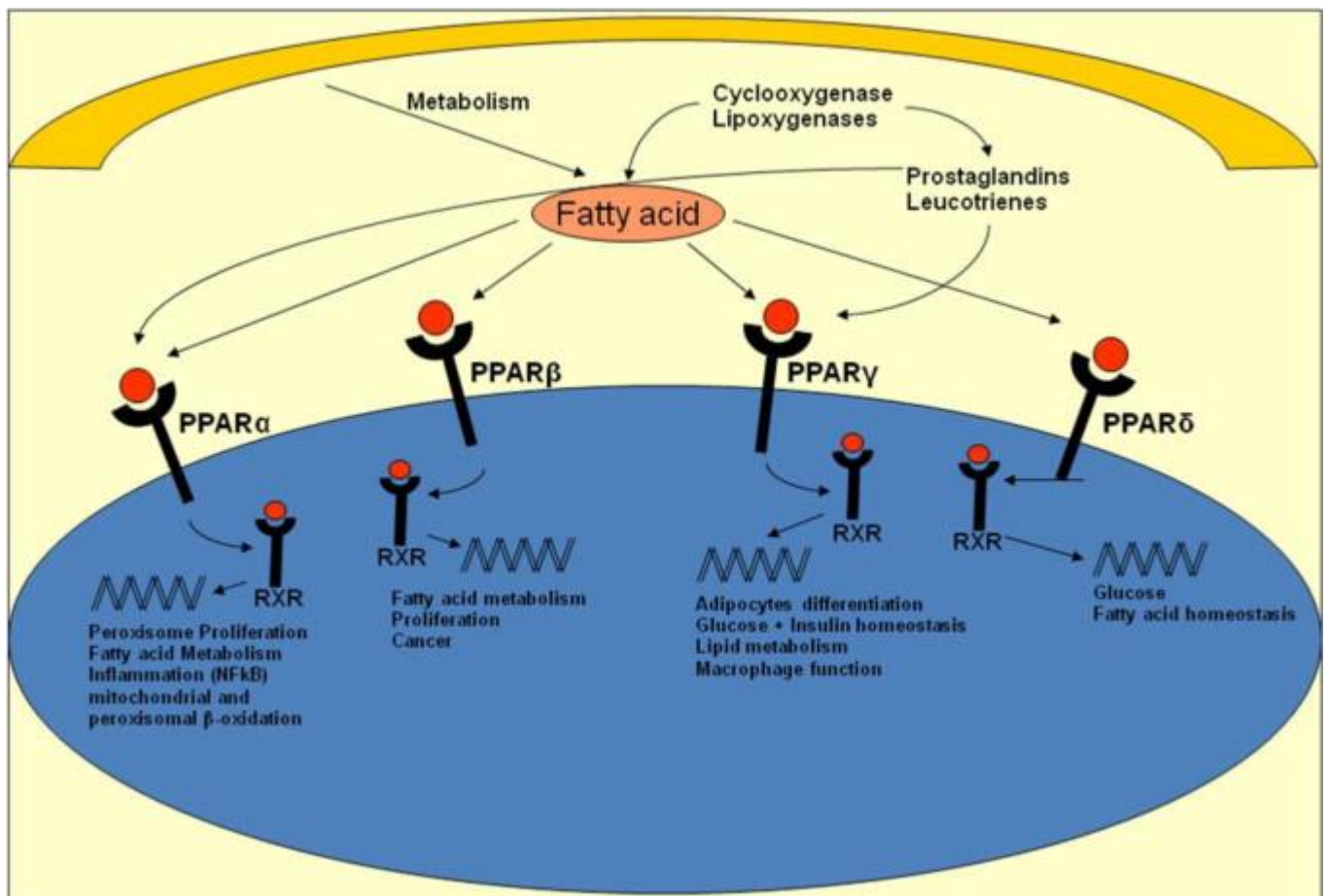


Fig. 4.

A summary of the different cellular effects of PPARs, associated to several aspects of cell metabolism and proliferation. Please see text for references.

PPAR α : The gene encoding PPAR α was initially cloned from mouse liver cDNA on the basis of its properties as a nuclear-receptor activated by carcinogens that induce peroxisome proliferation in the liver [113]. PPAR α , which recognizes monounsaturated and polyunsaturated fatty acids and eicosanoid is predominantly expressed in the liver, brown fat and heart, and has been implicated in regulating cellular energetics [93]. As such, many PPAR α target genes are involved in mitochondrial and peroxisomal β -oxidation of fatty acids [93]. Numerous studies of PPAR α $^{-/-}$ mice have shown that fasting or high-fat feeding of these mice results in abnormal lipid accumulation in hepatocytes, which is consistent with a crucial role for PPAR α in hepatic lipid

metabolism [114] and [115]. In addition, in normal rats, high-fat feeding-induced steatosis was related to PPAR α downregulation, presumably mediated by increased circulating free fatty acid [116].

PPAR α is expressed in human and mouse immune cells, including lymphocytes, macrophages and dendritic cells. Numerous studies have implicated PPAR α in the negative regulation of inflammatory responses. How PPAR α influences inflammatory gene expression remains incompletely understood. Several mechanisms are known to be involved, including direct interactions with the transcription factors NF- κ B and AP1, alterations in cytokine-receptor and growth factor receptor signalling, and up-regulation of expression of a subunit of inhibitor of NF- κ B (I κ B) [117]. Given that PPAR α regulates numerous genes and mechanisms involved in lipid metabolism and inflammation, it could be beneficial in the pathogenesis of NASH. Supplementation of n-3 polyunsaturated fatty acid, a PPAR α ligand, to HFD-treated animals restored hepatic adiponectin and PPAR α expression, reduced TNF α hepatic levels, and ameliorated fatty liver and the degree of liver injury [116]. In patients with NAFLD, supplementation with n-3 polyunsaturated fatty acid reduced hepatic steatosis and transaminase value [118]. Furthermore, in wild-type mice, HFD significantly increased the hepatic and adipose expression of numerous genes involved in inflammation. This effect was amplified in PPAR α -/- mice and reduced in wild-type by treatment with the PPAR α ligand Wy-14643, suggesting an anti-inflammatory role of PPAR α in liver and adipose tissue [115]. In agreement with this, in a model of NASH, tranilast (that inhibits the action of the fibrogenic transforming growth factor-beta, TGF- β) attenuated hepatic inflammation, down-regulated TNF- α expression, and up-regulated PPAR α [119]. Following high sucrose diet, a pharmacological IKK2 inhibitor attenuated the inflammatory response both in the adipose tissue and in the liver, and improved lipid β -oxidation mediated by PPAR α [120]. Liver-specific inactivation of the NF- κ B essential modulator gene (NEMO) synergizes with an HFD in the development of liver steatosis as a consequence of decreased PPAR α and increased PPAR γ expression. Steatosis interacts with increased inflammation, causing elevated apoptosis in the livers of these mice under HFD [121].

In other forms of chronic liver injury, the role of PPAR α is more than complex. Hepatic concentrations of PPAR α and its downstream target-gene CPT1A expressed by hepatocytes were impaired profoundly in the livers of untreated patients with HCV infection compared with controls [122]. In mice, liver-specific transgenic expression of the HCV core protein induced hepatic steatosis in all animals and HCC development in 35% of them [123].

PPAR γ : PPAR γ is expressed most abundantly in adipose tissue but is also found in pancreatic β cells, vascular endothelium, and macrophages. Its expression is low in tissues that express predominantly PPAR α , such as the liver, the heart, and skeletal muscle [124]. PPAR γ is essential for normal adipocyte differentiation and proliferation as well as fatty acid uptake and storage. The PPAR γ genetic program includes target genes involved in the uptake of glucose in muscle (c-Cbl associated protein and glucose transporter 4), lipid metabolism thus reducing the hepatic supply of fatty acids from adipose tissue and increasing β -oxidation by AMPK activation, and energy expenditure (glycerol kinase and uncoupling proteins 2 and 3) [93], [94], [124] and [125]. A range of naturally occurring ligands can activate PPAR γ , including unsaturated fatty acids, eicosanoids and components of oxidized LDLs. In addition, PPAR γ is the molecular target of thiazolidinediones, which sensitize cells to insulin and are in clinical use for their antidiabetic effects in the liver, adipose tissue and skeletal muscle. Possible mechanisms of PPAR γ -induced insulin sensitivity include increased lipid uptake and storage, leading to decreased free fatty acids and serum triglycerides, suppression of hepatic gluconeogenesis, and a small contribution toward increased uptake of glucose by adipose tissues [124].

Thiazolidinediones firstly promote fatty acid uptake and storage in adipose tissue. In this way, they increase adipose-tissue mass and spare other insulin-sensitive tissues such as skeletal muscle and the liver, and possibly pancreatic β cells, from the harmful metabolic effects of high concentrations of free fatty acids. By keeping fat where it belongs, thiazolidinediones increase hepatic insulin sensitivity (the ability of insulin to suppress endogenous glucose production) and insulin sensitivity in adipose tissue (measured from the ability of insulin to suppress free fatty acid concentrations) [126]. Thiazolidinediones might also modulate insulin sensitivity indirectly, by means of altered adipokine release able to regulate insulin sensitivity outside adipose tissue, such as resistin, adiponectin, and TNF- α [124].

In addition to transcriptional activation, an important function of PPAR γ is to inhibit inflammatory gene expression in a signal-specific manner. This process is termed transrepression because inhibition does not depend on the binding of PPAR-RXR heterodimers to PPREs in target-gene promoters. Numerous studies have shown that administration of PPAR γ ligands can ameliorate inflammatory responses in the pancreas, lungs, joints, nervous system and gastrointestinal tract, suggesting a therapeutic potential for PPAR γ agonists that selectively modulate inflammation [127]. In the liver, PPAR γ ligands have been shown to restore the quiescent phenotype of hepatic stellate cells in culture and to reduce activated stellate cell numbers in rodent models of fibrosis and NASH [128], [129] and [130]. All of these effects could reduce hepatic fat accumulation and related inflammation in NASH. This is probably the reason why thiazolidinediones have received great attention in the therapy of NASH. A recent proof-of-concept study showed a reduction in both hepatic fat and inflammation in diabetic NASH patients taking pioglitazone. In addition pioglitazone reduced insulin and free fatty acid levels, indicating improved insulin sensitivity in the peripheral and adipose tissue. Remarkably pioglitazone did not affect the degree of fibrosis, presumably due to the short period (6 months) of the study [131]. When the treatment with pioglitazone was extended to 12 months in non-diabetic NASH patients, a significant reduction in the degree of hepatic fibrosis was also observed [132]. On the other hand, a further recent using rosiglitazone, showed a reduction in insulin resistance and hepatic steatosis, without improvement in other histological parameters [133].

PPAR δ : PPAR δ (also known as PPAR β) is ubiquitously expressed, suggesting a fundamental requirement for PPAR δ signalling in many tissues [93] and [117]. Dietary fatty acids (FAs) carried by VLDL have been shown to activate PPAR δ . PPAR δ controls many metabolic programs in glucose and fatty acid homeostasis through direct transcriptional regulation, while its activity in suppressing inflammatory response is believed to be indirect. Pharmacological or genetic activation of PPAR δ exerts many therapeutic effects, including reducing hepatic glucose production, increasing fatty acid catabolism in adipocyte and muscle, lowering the atherogenic mechanisms [117]. In animal models, these effects lead to attenuation of metabolic syndrome, such as obesity, dyslipidemia and insulin resistance [134].

It has been proposed that the glucose lowering effect is mediated, in part, by metabolizing glucose for lipogenesis in the liver [135]. Thus, the lipogenic activity in the liver can be a concern as hepatic steatosis is common in diabetic patients. However the PPAR δ ligand GW501516 reduced fatty liver in high-fat fed C57BL/6 mice, and decreased hepatic TG and inflammation in a mouse model of nonalcoholic steatohepatitis (NASH) induced by a diet deficient in methionine and choline [123] and [136]. Collectively, these findings suggest that PPAR δ is a potential therapeutic target for insulin resistance and hepatic steatosis.

4. Conclusions

Nonalcoholic fatty liver disease (NAFLD) comprises a disease spectrum which includes variable degrees of simple steatosis (nonalcoholic fatty liver, NAFL), nonalcoholic steatohepatitis (NASH)

and cirrhosis. NAFLD can be considered the hepatic manifestation of the metabolic syndrome that recognizes insulin resistance as the main pathogenetic mechanism. Thus, NAFLD represents a risk factor for cardiovascular diseases. In addition, NAFLD represents the more common cause of chronic liver disease. However, no specific therapies for NAFLD, and its progressive form NASH, are currently available. In recent years, an outstanding number of studies have better defined the pathogenetic mechanisms that regulate the shift from NAFLD to NASH. On this regard, an exhaustive description of the cross-talk between visceral adipose tissue and the liver, in terms of adipokines, cytokines and growth factors, has been provided to explain the chronic inflammatory state associated to the metabolic syndrome. Adipokines, cytokines and growth factors can modify hepatic metabolism by acting on specific nuclear receptors that could represent the target of specific drug therapies.

Conflict of interest

None declared.

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